Left ventricular function in the post-infarct failing mouse heart by magnetic resonance imaging and conductance catheter: a comparative analysis


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Abstract

Background
Murine myocardial infarction (MI) models are increasingly used in heart failure studies. Magnetic resonance imaging (MRI) and pressure-volume loops by conductance catheter (CC) enable physiological phenotyping. We performed a comparative analysis of MRI versus CC to assess left ventricular (LV) function in the failing mouse heart.

Methods
MI was created by LAD ligation. MRI (day 14) and CC (day 15) were used to determine LV end-diastolic volume (EDV), end-systolic volume (ESV) and ejection fraction (EF).

Results
Pooled data yielded moderate-to-strong linear correlations: EDV: R=0.61; ESV: R=0.72; EF: R=0.81. We analyzed 3 groups, no MI (sham, n=10), small MI (<30% of LV, n=14), and large MI (>30%, n=20). Volumes and EF were consistently lower by CC than by MRI, but group differences were evident for both techniques. Receiver-operating characteristic (ROC) analysis indicated good sensitivity and specificity for both techniques, with superior results for MRI.

Conclusions
CC and MRI are highly valuable for evaluation of LV volume and function. MRI is recommended for longitudinal studies, accurate absolute volumes, and anatomic information. Unique features of CC are its online signal with high temporal resolution, and advanced analysis of LV function and energetics.
Introduction
Development and evaluation of new experimental therapies for heart failure increasingly involves functional analyses in mouse models. The most widely used model to study the failing heart and evaluate the effects of novel therapies is the myocardial infarction (MI) model. This model is typically created by permanent occlusion of the left anterior descending coronary artery (LAD). Currently, multiple methods are available to assess hemodynamics and ventricular function in the intact mouse. In the present study we compared two methods: magnetic resonance imaging (MRI) and pressure-volume loops by conductance catheter (CC) which both enable cardiac phenotyping under physiological conditions. Although these techniques are both increasingly used in heart failure studies no direct head-to-head comparison in the (chronic) post-infarct failing mouse heart model is yet available. In addition to comparison of the volumetric parameters which can be obtained by both methods we also illustrate and discuss the complementary additional features of MRI and CC and their value for heart failure research.

Methods
Animal model
Experiments were performed in 8- to 10-weeks-old male non-obese diabetic severe combined immunodeficient (NOD/scid) mice (Charles River Laboratories, Maastricht, The Netherlands) with average body weight of 25.3±3.0 g. All procedures were approved by the Animal Research Committee of the Leiden University and conformed to the Guide for Care and Use of Laboratory Animals (NIH publication No.85-23, Revised 1996).

Creation of the myocardial infarction
Myocardial infarctions were created as described previously. Briefly, animals were preanesthetized with 5% isoflurane in a gas mixture of oxygen and nitrogen (1:1) and placed supine on a heating pad (37°C). After intubation, ventilation was started (rate 200 breaths/min, stroke volume of 200 μL) using a Harvard Rodent Ventilator (Model 845, Harvard Apparatus, Holliston, MA, USA) with 1.5% isoflurane. We did not observe evidence of myocardial depression caused by isoflurane anesthetics. Subsequently, a left thoracotomy was performed in the fifth intercostal space, the pericardial sac was opened, and LAD was ligated permanently using a 7-0 prolene suture. The thorax was closed in layers and the animals were allowed to recover (MI group, n=39). In a control group the same operation was performed without LAD ligation (sham or ‘no MI’ group, n=10). The numbers represent the animals with complete data that survived the entire protocol until sacrifice after the pressure-volume study at day 15. The whole surgical procedure took ~45 minutes, with acute mortality <5%. There was no long-term mortality in the sham group and long-term mortality in the MI group was 25%. A time schedule of the experimental protocol is shown in Figure 1.
Magnetic resonance imaging
MRI was performed 2 days (infarction size) and 14 days (cardiac function) after surgery. We used a 9.4 T (400 MHz) Bruker BioSpin (Bruker, Etlingen, Germany) system with a 89 mm vertical bore, a shielded gradient set (1 T/m) and a rise time of 110 μs. A birdcage radiofrequency coil (Bruker) with inner diameter of 30 mm was used to transmit and receive the signals. A water-bath around the coil was kept at 29°C to achieve a rectal temperature of ~35°C. Before imaging, mice were anesthetized as described above and placed in a coil with a pneumatic pillow for respiration monitoring and maintained at 1.5-2% isoflurane guided by respiratory rate. ECG electrodes were attached to the left fore limb and right hind limb. ECG- and respiration-triggered image acquisition was performed using Bruker ParaVision 3.02 software.

On day 2, images were made 40±15 min after injection of the contrast agent Gadolinium-DOTA (Dotarem, Guerbet, Gorinchem, the Netherlands) via the tail vein to determine infarct size. To cover the entire left ventricle (LV), a multi-slice FLASH sequence (18 contiguous, 0.5-mm slices) was used with a 60° flip angle, 45 ms repetition time, 1.9 ms echo time, and 6 averages. On day 14, we performed single-slice cine FLASH imaging using 9 contiguous 1-mm thick slices covering the LV. Dependent on heart rate (HR), 18-26 frames were acquired per cardiac cycle. The cine FLASH sequences used a 15° flip angle, 7 ms repetition time, 1.9 ms echo time, and the signal was averaged 4 times. Pixel size was 100 x 100 μm (field of view was 25.6 x 25.6 mm, projected on a 256 x 256 matrix). All images were analyzed with MASS for Mice software. Epicardial and endocardial borders were delineated manually. Subsequently, LV end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) were computed automatically. Gadolinium provided clear contrast between infarcted (white) and non-infarcted myocardium (black) in the delayed enhancement images. Using a grayscale threshold value the enhanced region was automatically detected and the infarcted area calculated (Figure 2). The appropriate threshold value was selected manually based on a histogram of grayscale values from the area between the endocardial and epicardial contours and confirmed by visual inspection of the detected infarct area.
Chapter 7
MRI and CC to assess failing mouse heart

Left ventricular pressure-volume loops by conductance catheter
On day 15, the animals were again anesthetized as described above. The animals were placed supine on a warming mat under a surgical microscope. Via the right carotid artery, a 1.4 F pressure-conductance catheter (SPR-719, Millar, Houston, TX, USA) was positioned into the LV. The catheter used in this study contains four platinum electrodes, each 0.25 mm in width with inter-electrode distances of 0.5, 4.5 and 0.5 mm between electrodes 1-2, 2-3, and 3-4, respectively. A high-fidelity solid state pressure sensor is incorporated between electrodes 2 and 3. A 30 μA, 10 kHz current is applied between electrodes 1 and 4 to generate an intracavitary electric field and the voltage gradient between electrodes 2 and 3 is measured to determine the instantaneous electrical conductance of the blood in the LV (Figure 3). Catheter positioning was guided by online pressure and volume signals: After passing the aortic valve as indicated by the pressure signal, the catheter was pushed forward to wedge into the LV apex. Subsequently the position was optimized by inspection of the volume signal aiming at a maximal stroke volume. The abdomen was opened to enable preload reductions by compressing the inferior caval vein. The CC was connected to a Sigma-SA signal-processor (CD Leycom, Zoetermeer, The Netherlands) for online display and registration of LV pressure and volume signals. Parallel conductance was obtained by the hypertonic saline method using intravenous bolus injections of ~5 μL. Slope factor $g$ was obtained using standardized volumetric calibration cuvettes with bore diameters of 3-7 mm as described by Yang et al. Data were acquired with Conduct-NT software (CD Leycom) at a sample rate of 2000 Hz and analyzed offline with custom-made software.

Pressure-volume signals were acquired in steady state to obtain HR, cardiac output (CO), EDV, ESV, EF, end-diastolic pressure (EDP), end-systolic pressure (ESP), stroke work (SW), $dP/dt_{\text{max}}$ and $-dP/dt_{\text{MIN}}$ and isovolumic relaxation time constant Tau. Load-independent indices of systolic and diastolic LV function were determined from pressure-volume relations obtained during preload reductions: the end-systolic pressure-volume relation (ESPVR) and the preload recruitable stroke work relation (PRSWR: SW versus EDV). The slopes of these relations (end-systolic elastance $E_{\text{es}}$ and $S_{\text{PRSW}}$, respectively), and their intercepts ($ESV_{\text{ESPVR,INT}}$ and $EDV_{\text{PRSW,INT}}$) are sensitive measures of intrinsic systolic LV function. For diastolic function, the chamber stiffness $E_{\text{es}}$ was determined from a linear fit to the EDP-volume points.

Figure 2: Assessment of infarct size by delayed enhancement 2 days after myocardial infarction. Epicardial and endocardial contours were manually traced (left panel). Infarct area was then automatically detected using a grayscale threshold value (right panel).
Statistical analysis
Data were analyzed using SPSS 11.0 software (SPSS Inc, Chicago, IL, USA). To compare MRI and CC-derived indices we performed linear regressions, calculated correlation coefficients, and performed Bland-Altman analyses. Receiver-operating characteristic (ROC) curve analyses were used to determine the optimal cut-off values to discriminate groups and calculate the corresponding sensitivities and specificities. Independent multiple t-tests were performed as post hoc test if one-way ANOVA demonstrated significant differences. Data are reported as mean±SD. A value of p<0.05 was considered to indicate significant differences.
Results

EDV, ESV, and EF as determined by CC and MRI showed moderate-to-strong correlations, although CC-derived values were systemically lower than those by MRI. Orthogonal linear regression results of all pooled data (n=49) are shown in Figure 4. Bland-Altman analyses on the pooled data confirmed the underestimation by the CC showing a bias of -28±29 μL for EDV, -18±27 μL for ESV and -8±8% for EF.

For further analysis, the MI mice were divided into groups with small (<30% of LV) or large (>30%) MI size as determined by the MRI on day 2. Mean infarct sizes in the small MI (n=14) and large MI (n=20) groups were 27±3% and 37±5%, respectively. In 5 mice no MI size could be determined because no successful Gadolinium injection was performed and they were therefore excluded from this part of the analysis (they were, however, included in the analysis of the pooled data as shown in Figure 4). Figure 5 shows mean EDV, ESV and EF for both methods in all groups. Results indicated that the mean EDV and ESV were almost identical between techniques in the non-infarcted hearts, but that CC-derived volumes were smaller in the MI groups. Interestingly, EF was consistently higher for all groups when measured with MRI (Figure 5).

As shown in Table 1, both methods detected significant differences for all parameters both when comparing sham-operated (no MI) versus small MI and when comparing small MI versus large MI, except that CC-derived EDV did not reach statistical significance (p=0.084) in the latter comparison. ROC curve analyses determined the optimal cut-off values to discriminate groups and the corresponding sensitivities and specificities. The results indicated that MRI is the best method to distinguish different groups based on measured volumes and EF. For distinction between sham and small MI groups, MRI-derived cut-off values could be chosen with 100% sensitivity and specificity. Optimal CC-derived cut-offs resulted in a sensitivity of 93% and specificity of 80% for ESV and EF, and in a sensitivity of 73% and specificity of 80% for EDV. For comparison of small versus large MI groups, MRI again demonstrated better results for EDV and ESV, whereas EF showed comparable diagnostic accuracy with both techniques.

To illustrate the effect of MI on LV size and geometry, Figure 6 shows typical examples of short-axis MRI views. Wall thinning indicating the infarct area and enlarged LV leading to reduced EF are clearly visible. Whereas MRI is currently regarded as the gold standard method for visualizing the heart in small animals, the CC has unique features for determining the functional effects of MI. Figure 7 shows the schematic average pressure-volume loops (based on mean EDV, ESV, EDP and ESP) for the three groups. The figure clearly shows the gradual deterioration of LV function evidenced by increased LV volumes, reduced SV (loop width) and SW (loop area), reduced ESP and increased EDP. A more detailed analysis of the functional effects is presented in Table 2. When comparing the small MI versus the sham group, general hemodynamics show a maintained CO but reduced SW. Systolic function is clearly depressed evidenced by a significantly increased ESV, and decreased EF and dP/dt\text{MAX}. Moreover, ESPVR and PRSW show a significant rightward shift as illustrated by the increase of their intercepts, indicating a significant decrease in systolic myocardial function. EDV and ESP are both increased, whereas -dP/dt\text{MIN} was significantly reduced. At this stage, however, Tau and diastolic stiffness (E\text{ed}) were not yet altered, indicating that intrinsic diastolic function was largely maintained and that the increases in EDP and EDV mainly represent changes in loading, which enable the LV to maintain CO by using its Starling mechanism. The functional decline is much more profound in the large MI group: General hemodynamics are significantly depressed as indicated by a further 40% decline in CO and almost 50% decrease in SW. The systolic indices indicate a further significant decrease in LV function and, at this stage, diastolic LV function is also impaired (although Tau only reached marginal significance). The 88% increase in diastolic stiffness clearly hampered the LV to further employ the Starling mechanism to compensate for the loss in systolic function.
Figure 4: End-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) by magnetic resonance imaging (MRI) and by conductance catheter (CC). MRI data were obtained 14 days after myocardial infarction (MI), CC data 15 days after MI. Comparison was made by linear regression (left panels) and by Bland-Altman analyses (right panels). In the Bland-Altman plots, bias and limits of agreement (LoA = bias ± 2 SD) are indicated by horizontal dotted lines.
Figure 6: Example of magnetic resonance images obtained at day 14. Short axis view of a sham-operated heart (no myocardial infarction, MI), a heart with small MI and a heart with large MI. Note the wall thinning in the anterior left ventricular (LV) wall (small MI) or the entire LV wall (large MI), indicating the infarct area. EDV: end-diastolic volume, ESV: end-systolic volume.

Figure 5: Mean end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) by magnetic resonance imaging (MRI) and conductance catheter (CC) in mice without myocardial infarction (MI; sham), small (<30%) and large (>30%) MI. MRI data were obtained 14 days after MI, CC data 15 days after MI. Significances between groups: * p<0.01, ** p<0.001. Significances between methods: # p<0.05, ## p<0.001. Detailed statistics are presented in Table 1.
Figure 7: Schematic pressure-volume loops (based on mean end-diastolic and end-systolic pressures and volumes) obtained by conductance catheter in sham-operated mice without myocardial infarction (MI), and mice with small and large MI. LV: left ventricular.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>Small MI</th>
<th>Large MI</th>
<th>P</th>
<th>Cut-off</th>
<th>Sens</th>
<th>Spec</th>
<th>AUC</th>
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<td>MRI</td>
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<tr>
<td>EDV (μL)</td>
<td>51±5</td>
<td>97±22</td>
<td>127±26</td>
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<td>61</td>
<td>100%</td>
<td>100%</td>
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<td>ESV (μL)</td>
<td>25±4</td>
<td>71±24</td>
<td>104±29</td>
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<td>36</td>
<td>100%</td>
<td>100%</td>
<td>1.00</td>
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<td>EF (%)</td>
<td>52±4</td>
<td>28±8</td>
<td>19±7</td>
<td>&lt;0.001</td>
<td>42</td>
<td>100%</td>
<td>100%</td>
<td>1.00</td>
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<td>CC</td>
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<tr>
<td>EDV (μL)</td>
<td>50±16</td>
<td>75±17</td>
<td>87±20</td>
<td>&lt;0.002</td>
<td>63</td>
<td>73%</td>
<td>80%</td>
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<td>ESV (μL)</td>
<td>32±11</td>
<td>58±16</td>
<td>76±21</td>
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<td>41</td>
<td>93%</td>
<td>80%</td>
<td>0.93</td>
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<td>EF (%)</td>
<td>36±9</td>
<td>24±7</td>
<td>13±6</td>
<td>&lt;0.001</td>
<td>31</td>
<td>93%</td>
<td>80%</td>
<td>0.83</td>
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Table 1: Left ventricular end-diastolic volume (EDV), end-systolic volume (ESV) and ejection fraction (EF) by magnetic resonance imaging (MRI) and conductance catheter (CC) in mice without myocardial infarction (MI; Sham), with small MI (<30% of LV), or large MI (>30%) at day 14 (MRI) or day 15 (CC). Receiver-operating characteristic (ROC) analysis to determine optimal cut-off, sensitivity (Sens), specificity (Spec) and area under the curve (AUC). Significant differences between corresponding values obtained by MRI and CC within the same group: # p<0.05, ## p<0.001.
<table>
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<tr>
<th>Groups</th>
<th>Sham</th>
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<th>Large MI</th>
<th>Sham vs. Small MI</th>
<th>Small vs. Large MI</th>
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<td><strong>General</strong></td>
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<tr>
<td>HR (beats/min)</td>
<td>424±50</td>
<td>438±45</td>
<td>452±62</td>
<td>0.498</td>
<td>0.476</td>
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<td>CO (mL/min)</td>
<td>8.6±3.3</td>
<td>7.9±2.9</td>
<td>4.7±1.9*</td>
<td>0.620</td>
<td>&lt;0.001</td>
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<td>SW (mmHg.mL)</td>
<td>1.76±0.69</td>
<td>1.07±4.9*</td>
<td>0.55±0.30*</td>
<td>0.009</td>
<td>0.001</td>
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<tr>
<td>E_s (mmHg/μL)</td>
<td>5.5±4.2</td>
<td>4.8±1.4</td>
<td>8.2±2.9*</td>
<td>0.572</td>
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<td>E_max / E_s</td>
<td>0.58±0.32</td>
<td>0.56±0.28</td>
<td>0.30±0.11*</td>
<td>0.860</td>
<td>0.004</td>
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<td><strong>Systolic</strong></td>
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<tr>
<td>ESV (μL)</td>
<td>32±11</td>
<td>58±16*</td>
<td>76±21*</td>
<td>0.001</td>
<td>0.009</td>
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<td>ESP (mmHg)</td>
<td>89±20</td>
<td>81±16</td>
<td>77±14</td>
<td>0.303</td>
<td>0.398</td>
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<tr>
<td>EF (%)</td>
<td>36±9</td>
<td>24±7*</td>
<td>13±6*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>dP/dt_max (mmHg/ms)</td>
<td>8.2±1.8</td>
<td>6.2±2.5*</td>
<td>4.6±1.7*</td>
<td>0.036</td>
<td>0.033</td>
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<td>E_max (mmHg/μL)</td>
<td>3.0±2.0</td>
<td>2.5±1.2</td>
<td>2.3±0.7</td>
<td>0.461</td>
<td>0.648</td>
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<td>ESV_ESPVR,INT (μL)</td>
<td>29±9</td>
<td>52±19*</td>
<td>74±20*</td>
<td>0.001</td>
<td>0.008</td>
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<td>S-PRSW (mmHg)</td>
<td>57±21</td>
<td>53±12</td>
<td>36±19*</td>
<td>0.577</td>
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<td>EDV_ESPVR,INT (μL)</td>
<td>35±7</td>
<td>71±19*</td>
<td>95±23*</td>
<td>&lt;0.001</td>
<td>0.009</td>
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<td><strong>Diastolic</strong></td>
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<tr>
<td>EDV (μL)</td>
<td>50±16</td>
<td>75±17*</td>
<td>87±20</td>
<td>0.002</td>
<td>0.084</td>
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<td>EDP (mmHg)</td>
<td>6.6±3.1</td>
<td>12.4±7.3*</td>
<td>14.8±6.6</td>
<td>0.025</td>
<td>0.329</td>
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<td>-dP/dt_min (mmHg/ms)</td>
<td>5.8±1.2</td>
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<td>Tau (ms)</td>
<td>14.8±4.0</td>
<td>16.5±5.4</td>
<td>20.8±7.5</td>
<td>0.409</td>
<td>0.085</td>
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<tr>
<td>E_max (mmHg/μL)</td>
<td>0.61±0.58</td>
<td>0.59±0.32</td>
<td>1.11±0.81*</td>
<td>0.936</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Table 2: Conductance catheter-derived functional indices for sham-operated (no MI), small MI and large MI mice at day 15. MI: myocardial infarction, HR: heart rate, CO: cardiac output, SW: stroke work, E_s: effective arterial elastance, E_max: end-systolic ventricular elastance, ESV: end-systolic volume, ESP: end-systolic pressure, EF: ejection fraction, dP/dt_max: maximal rate of LV pressure increase, ESV_ESPVR,INT: intercept of the ESPVR (end-systolic pressure-volume relation) at ESP=81 mmHg (overall mean), S-PRSW: slope of preload recruitable stroke work relation (PRSW), EDV_ESPVR,INT: intercept of the PRSW at EDV=74 μL (overall mean), EDV: end-diastolic volume, EDP: end-diastolic pressure, -dP/dt_min: maximal rate of LV pressure decline, Tau: relaxation time constant, E_max: end-diastolic stiffness. * indicates significance of small MI group versus sham group; # indicates significance of large MI group versus small MI group.
Discussion

The rationale for our critical evaluation of functional measurements in the murine MI model is found in the extensive application of this model in current cardiovascular research. Both MRI and CC are widely used to assess LV function in such studies, but no direct comparative analysis is yet available for the post-infarct failing mouse heart. We demonstrated for the first time in a mouse model with vast LV remodeling that LV volumetric indices obtained by MRI and CC are strongly correlated and that both methods reliably detect changes in LV volumes and EF. In addition, the two techniques yield highly complementary information. A previous study in the normal mouse heart also indicated a strong linear correlation between MRI- and CC-derived volumes. In addition, in line with our findings, these authors showed that CC-derived volumes underestimated MRI-derived volumes. However, in our study this underestimation was not present in the sham (no MI) animals, but only in the infarct groups with a larger EDV. An earlier study demonstrated an even more pronounced underestimation which could be partly due to a larger baseline MRI-derived EDV (79±8 versus 51±5 μL in our study) consistent with a higher body weight (29±4 versus 25±3 g). However, differences could also be related to the methods used for CC calibration. Unfortunately, this study only presented mean values and the correlation between MRI-derived and CC-derived volumes was not investigated. The authors repeated their measurements in mice with LV hypertrophy induced by aortic constriction. Compared with control, the hypertrophic animals showed increased LV volumes with both methods; however, with MRI the increase was largest in EDV and with CC the increase was mainly in ESV. This resulted in an observed increase in stroke volume with MRI and a tendency for a decrease with CC, whereas EF was unchanged with MRI and decreased with CC. Based on these findings the authors concluded that directional changes induced by aortic constriction obtained by CC are inaccurate. Obviously, this conclusion can only be drawn based on the assumption that MRI represented the gold standard. Although this is generally assumed, the reliability of MRI to assess LV volumes in the hypertrophic mouse model has not been directly demonstrated and was based mainly on accuracy of LV mass estimates versus autopsy data. In fact, from a physiological standpoint the CC-derived decrease in CO and EF may be more plausible than the opposite changes found with MRI: previous (echocardiographic) studies in similar models typically showed reduced SV or CO, unchanged LV end-diastolic diameters and reduced fractional shortening in line with the CC-derived findings. Thus, further studies may be needed to assess the reliability of both MRI and CC in the (non-failing) hypertrophic mouse model.

It should be noted that in all previous comparative studies, including ours, CC- and MRI-derived volumes were not obtained simultaneously, and that conditions in the two measurement sessions were generally partly different. In all cases, CC measurements were obtained after the MRI assessments which, particularly in failing heart models, may have resulted in more depressed LV function in the later session after repeated anesthesia. In our study both sessions were performed in closed-chest conditions. The abdomen was opened during the CC measurements for performing caval occlusions, however, this would not be expected to have important hemodynamic consequences since intra-thoracic pressure was not disturbed. However, Nielsen et al. introduced the CC via the apex after thoracotomy. The effects of opening the chest may be difficult to predict and is dependent on anesthesia, potential blood loss and fluid supplementation. In general, lower pressures and absolute volumes could be expected but reductions in SV and CO may be limited due to simultaneous reduction in afterload. In addition, MRI was generally performed during spontaneous breathing, whereas CC measurements were done after intubation and with artificial ventilation. These different protocols may have resulted in different levels of anesthesia as suggested by HR differences. Most studies report higher HR during CC sessions. Moreover, the CC measurements were generally performed supine, in contrast to a vertical head-up position with MRI. The latter position may be associated with a lower CO and EDV particularly in failing hearts, but the differences presumably are small. Although due to these factors absolute volumes may not be completely comparable
during sequential MRI and CC sessions, the finding of relative underestimation by CC appears to be fairly consistent, particularly for larger LV volumes.

Several factors may have contributed. With regard to the CC method, LV volume is measured in one single segment defined by the distance between the sensing electrodes (4.5 mm) which, particularly in an extremely dilated LV, may largely explain the observed underestimation. Furthermore, issues related to calibration of the CC may have played a role. In our study the slope factor $\alpha$ was determined in vitro by placing the CC in different sized cylinders with diameters ranging from 3 to 7 mm. The calibration curve was highly linear with a slope $\alpha = 0.29\pm0.02$ and correlation $r^2 = 0.986$, but theoretically the relation between true volume and conductance becomes non-linear if the diameter is large compared to the distance between the current electrodes (5.5 mm). This would be the case for extremely dilated LVs in the present study, and result in underestimation. Furthermore, parallel conductance (mean value $31\pm5 \mu L$) was assessed by intravenous hypertonic saline injections whereas, ideally, pulmonary artery injections should be used. Consistent with earlier studies in sheep, a recent study in mice showed a tight correlation between parallel conductances obtained from pulmonary artery and intravenous injections, but the latter consistently produced slightly higher values. As parallel conductance is subtracted from the raw measured conductance, this could also have contributed to the underestimation of volumes by CC compared to MRI.

MRI is considered the gold standard for absolute LV volume measurements as it does not require geometric assumptions, and excellent agreement with autopsy data was demonstrated. Some limitations, however, should be mentioned. Agreement with autopsy data was obtained for LV wall mass and to infer accuracy of LV cavity volumes may not be fully justified. The temporal resolution of MRI in the present study was 7 ms which at a typical cardiac cycle length of 150 ms corresponds to 21 frames/beat. Considering that the relaxation time constant was in the range of 15-20 ms (Table 2), at least two subsequent frames are obtained in the isovolumic relaxation period and thus the temporal resolution should be sufficient to accurately determine minimal LV volume (ESV). The situation at end-diastole is more critical because the isovolumic contraction period generally is shorter than the isovolumic relaxation period. Moreover, in case of mitral insufficiency, which presumably was present in some of the dilated hearts, substantial decreases in LV volume may occur before aortic valve opening, resulting in a very short isovolumic period. Thus, the limited temporal resolution of MRI could result in underestimation of true maximal volume.LV volume was calculated by summation of multiple slices and thus the spatial resolution of MRI was partly determined by slice thickness which was set at 1 mm in our study. To avoid discontinuous phasic volume signals we included only slices that showed the LV cavity throughout the cardiac cycle. This could have resulted in underestimation of EDV, because in practice the number of included slices was determined by ESV. Another limitation is related to the fact that MRI images are reconstructions based on data acquired over a large number of cardiac cycles. Particularly in the failing heart, rhythm disturbances and respiratory variability could result in gating problems and, subsequently, relatively long acquisition times which combined with inherent hemodynamic instability might affect image quality and reduce the accuracy and reproducibility of the data. Despite these (theoretical) limitations, image quality was adequate to reliably trace the endocardial borders in all animals.

The significant correlation between MRI- and CC-derived volumes and EFs indicated that both methods can be used to detect changes in LV volume. However, as the absolute values of the volumetric indices were not the same, the methods are not interchangeable. This conclusion is not surprising and in line with clinical studies comparing imaging modalities. Bland-Altman analyses confirmed the underestimation by the CC method and showed that underestimation was more pronounced at higher volumes but that variability remained fairly constant; however, as the volumetric values obtained by the two methods were not expected to be interchangeable we feel
that comparison by linear regression analysis is more insightful. To quantify the diagnostic accuracy of MRI and CC we determined sensitivity and specificity of the volumetric indices. The results were good for both methods, but MRI was clearly superior. It should be noted that this analysis implicitly assumes a strong correlation between infarct sizes and subsequent cardiac enlargement. The smallest infarct size in our study was 21%, which according to previous studies causes a drop in EF of at least 15-20% and thus no overlap in real volumes between the group without MI and the small MI group would be expected. However, the small MI and large MI groups were created retrospectively with small MI ranging from 21% to 30% and the large MI ranging from 31% to 47%. Thus some overlap of absolute volumes between groups and therefore no 100% sensitivity and specificity should be expected. Despite this, the ROC analyses indicated excellent results for MRI and good results for CC, particularly for CC-derived EF with 85% sensitivity and 87% specificity.

Finally, MRI and CC each have distinct advantages and disadvantages that should be considered. The non-invasive character of MRI is an important advantage which enables longitudinal studies in individual animals under physiological conditions. In particular, serial MRI studies are highly valuable to investigate the time course of ventricular remodeling after MI. The use of contrast agents such as Gadolinium in conjunction for cardiac MRI provides for the accurate assessment of infarct size early after MI. In addition, advanced techniques of cardiac MRI such as myocardial tagging can be used to assess regional contractile function in the mouse heart over time after MI. Regional measurements may also be used to determine infarct size by considering the myocardial portion with significant thinning and akinesia or dyskinesia during systole. Moreover, magnetic resonance may also be used for spectroscopy (MRS) to study metabolism and energetics of the heart. As a disadvantage, MRI studies are time-consuming both for data acquisition and for data analysis. Moreover, the equipment is expensive, requires considerable infrastructure and highly trained personnel. As a consequence, availability is generally limited.

The CC method, on the other hand, not only provides an instantaneous volume signal but by combining this with instantaneous LV pressure, pressure-volume relations can be obtained which are generally regarded as the gold standard for assessing LV systolic and diastolic function because these relations yield highly sensitive, load-independent indices. In the present study this was illustrated in Table 2 which shows, for example, that although when going from sham (no MI) to small MI to large MI the volumetric changes are largest at the first step, the functional effects particularly regarding intrinsic diastolic function and cardiac output are much more pronounced in the second step. Moreover, CC measurements give insight in the underlying mechanisms by providing detailed information on the extent of systolic and diastolic dysfunction and have possibilities for advanced analysis of mechanics and energetics of the heart and its interaction with the vascular system. In addition, the temporal resolution of the pressure-volume signals is high (0.5 ms in our study) and its continuous, online (beat-to-beat) nature enables dynamic studies which are not feasible with imaging techniques which require steady states. As a disadvantage, the CC is highly invasive and is generally only performed as a terminal study, although recently the feasibility of repeated measurements in conscious mice has been demonstrated.

In conclusion, our study indicates that CC and MRI are both methods that are highly valuable for evaluation of LV volume and function in murine MI studies. Selection of the optimal method depends on the specific research question: MRI is recommended for longitudinal studies, for accurate absolute volumetric measurements, and when anatomical information is essential. The unique features of the CC method are its online signal with high temporal resolution, and the possibilities for advanced analysis of LV function, mechanics and energetics.
Reference List


